Highly active anti-retroviral therapy significantly suppresses viral replication, but does not lead to complete eradication of HIV. Here the authors discuss a new model of viral decay, which in addition to the half-life of the viral resevoirs, takes into account infection bursts and immune activation events.

Ongoing HIV dissemination during HAART

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Multiphasic HIV decrease in individuals treated with anti-retroviral drugs has been modeled as the independent decay, with different half-lives, of distinct pools of cells infected before the initiation of treatment. We analyzed the kinetics of plasma HIV RNA in individuals receiving combinations of up to five drugs. The initial rates of decline increased substantially with the efficacy of treatment. Decline rates decreased with time, approaching zero in some cases. These observations are better explained if most of the virus is produced by cells infected after the initiation of therapy. Accordingly, treatment results in ongoing HIV infection cycles of decreasing amplitude, but the decrease progressively attenuates and may cease altogether at some viral load. We propose that HIV replication occurs in multiple local bursts, associated with immune activation in response to antigens. Current anti-retroviral drugs substantially reduce the size of these bursts and diminish their frequency but fail to abolish them.

The infected-cell half-life model

Drugs that inhibit *de novo* infection of cells in HIV-infected persons, without blocking virus production by previously infected cells, cause rapid decrease in the blood concentration of HIV-RNA of up to 100-fold in the first 1–2 weeks after treatment initiation (phase I), indicating that most of the virus was produced by short-lived infected cells[1,2]. This is followed by a slower decrease (phase II). After an initial delay of 1–2 days, the observed phase I decrease seemed exponential. This has been interpreted to reflect exponential death kinetics of previously infected, short-lived $CD4^+$ T cells, with the rate of decrease corresponding to the half-life of these cells, estimated to be about 1 day[3]. The slower phase II decrease was interpreted as directly reflecting the parallel but independent decay of pre-existing, longer-lived, infected cell populations (half-lives of 1–4 weeks)[4]. This allowed the estimation of the time required for complete eradication of HIV (ref. 4).

We challenged these interpretations, arguing that the death of productively-infected $CD4^+$ T cells is better characterized by a variable time delay than a half-life[5]. The delay corresponds to the interval from infection of a cell to its death. We argued that the progression of the productively-infected lymphocyte from infection to death should be likened to an 'aging' process, in which death probability sharply increases when a certain age is reached. Viral cytopathicity, anti-HIV cytotoxic T lymphocytes (CTLs), or activation-associated apoptosis cause cell death at that stage. If our proposition is correct, the kinetics of viral load in phase I cannot simply reflect death of previously infected cells.

Our data support these theoretical considerations. We determined normalized mean blood HIV RNA levels for 16 subjects, over a 6- to 7-day period from the initiation of treatment with four anti-retroviral drugs (Fig. 1). The changes in viral RNA are complex, but an analysis showed a 3.3-fold drop from day 2 to day 3. This would translate to a half-life of 14 hours, which is too short for completion of the intracellular infection cycle[6,7]. Similar rapid decreases in viral load have been inferred from *in situ* studies[8]. In contrast, fitting an exponential decay slope to the measured HIV-RNA decrease between days 3 and 6 gives a half-life of 41 hours (Fig.1). It is not obvious why the death of infected cells should be substantially slower during days 3–6 than during days 2–3. Thus, a different explanation is needed.

A second line of evidence is the large differences in mean decay rates in response to different treatment regimens. We determined rates for individuals treated with one, three or five anti-retroviral drugs (Fig. 2). The rates of decay during the first week, using a simple exponential approximation for this period, $\sim\exp(-\lambda t)$, were $\lambda = 0.18$/day, 0.41/day and 0.57/day, respectively, for these regimens. Furthermore, among children treated with protease inhibitors, the initial rate of decrease correlated with the nadir of plasma drug concentration[9]. If the decay rate reflected the rate at which previously infected cells died, it would not be expected to be related to treatment regimen or plasma drug concentration.

Ongoing viral replication

The dependence of the decay rate on treatment regimen and plasma drug concentration is predicted by assuming ongoing viral-infection cycles with reduced infection efficiency[5]. If the average reproduction ratio (R; Appendix 1) becomes smaller than 1, each cohort of productively-infected cells is replaced by a smaller number of cells in the subsequent cycle. As long as R and the average infection cycle time (τ) do not change, viral load will decay exponentially (apart from transients) according to $V = V(0)\exp(-\lambda t)$. The rate constant $-\lambda$ is $\sim\ln(R)/\tau$ (Appendix 2). In this approximation for λ, we neglected the very short half-life of free virus[10,11] and assumed a fixed τ for productively infected T cells, neglecting the variability of τ about its average. In this approximation, HIV kinetics are completely different if *de novo* infection is blocked completely (abrupt fall) or only partially (exponential decrease).

Even if R does not remain constant, which is already evident from Fig.1, the above expressions can be used in a piecewise exponential approximation of a changing slope. In comparing two different regimens, characterized respectively by R_1 and R_2 during a comparable period of time, the difference between the viral load decrease slopes, $\lambda_1-\lambda_2$, is approximated by $(1/\tau)\ln(R_2/R_1)$. The difference in slope is thus a direct measure of the relative treatment efficacy. This rationalizes phase I slope difference as a chief predictor of the virological and clinical outcome of therapy[9]. The lifespan of most in-

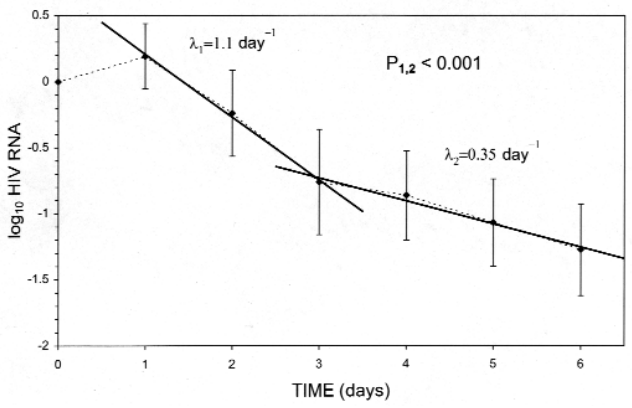


Fig.1 Plasma HIV RNA first-week decrease with four-drug combination therapy. Each time point represents the geometric mean of HIV RNA from 16 patients normalized to their baseline values. Bars, standard deviations. The solid lines were obtained by linear regression analysis. $P < 0.001$, λ_1 compared with λ_2.

fected cells τ was estimated from the 'shoulder' of the virus-concentration decay curves to be about 1.25 days[5].

Our interpretation differs fundamentally from that accepted at present. We explain the kinetics entirely in terms of changes in numbers of cells infected after the introduction of drugs, not before. Although current models allow for incomplete blocking of viral transmission, this is usually accommodated only as a small correction to exponential cell death kinetics (Appendix 2). There is a fundamental difference in the way these divergent interpretations are extended to phase II.

Multiphasic HIV decrease as a single process

The rate of viral-load decrease depends on the average τ and the average R. These phenomenologic parameters hide considerable microscopic complexity and change over time. Increase in either τ or R would decrease the slope of viral decay, providing an alternative interpretation of phase II and beyond.

To see how such changes could occur, we explored the close

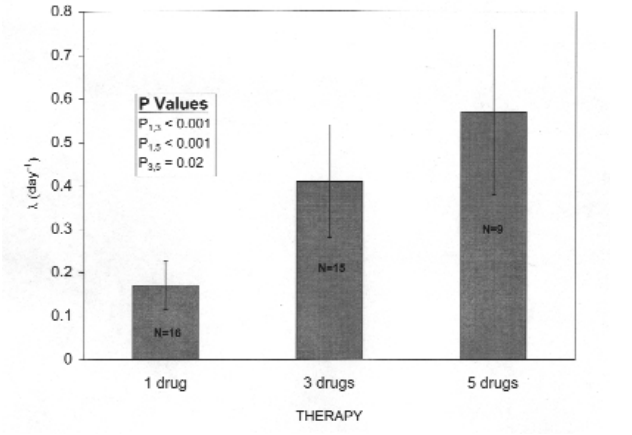


Fig.2 HIV RNA decay rate constants for one-, three- and five-drug regimens; the means of individual decay rate constants were calculated for 16, 15 and 9 patients, respectively. Bars, standard deviations. $P < 0.001$, one-drug compared with three-drug or five-drug; $P = 0.02$, three-drug compared with five-drug.

relationship between immune activation and HIV replication. We have argued that efficient virus transmission from infected to uninfected cells occurs only at close range, among immunologically co-activated cells[12]. Immune response episodes are local and transient, and therefore HIV is produced in intermittent bursts, initiated by the activation of a latently infected $CD4^+$ T cell or of an infectious antigen-presenting cell (APC) and terminated when the local response subsides. Systemically, virus production is continuous, because local bursts are multiple, overlapping and asynchronous. Immune activation in infected individuals occurring as multiple transitory immune responses is consistent with BrdU-labeling kinetics in SIV-infected macaques[13]. *Proximal activation and transmission*[12] is supported by studies of HIV variant distribution in lymphoid tissue which showed that distinct variants populate neighboring microscopic sites[14].

We propose that phase I decline mainly reflects rapid reduction in the magnitude of virus production bursts by drugs that only partially block *de novo* infection. The subsequent, progressively slower decrease mainly reflects the effect of the drugs on the frequency of new bursts.

Phase I: rapid fall of infected-cell burst size

Broadly, an immune response episode includes activation of resting lymphocytes by APCs; cytokine-driven amplification of responding-cell numbers near the activation sites; and finally, contraction of the responding populations due to activation-associated cell death and anergy. A fraction of the activated cells return to rest to become memory cells. When HIV-infected cells participate in such local events, cell activation supports virus dissemination. Infectious virus from productively infected $CD4^+$ T cells, infected macrophages and virus-bearing dendritic cells is transmitted to appropriately activated lymphocytes. If a latently infected $CD4^+$ T cell is activated by an APC, or an infectious APC activates a $CD4^+$ T cell, both partner cells may directly infect other activated $CD4^+$ cells and APCs, thereby initiating the process of local virus dissemination. Infection is then amplified as a chain-reaction in conjunction with amplification of the number of responding antigen-specific lymphocytes and 'bystanders' (Fig.3). Such amplification is transient and mostly site- and/or clone-restricted[12,15].

What is the impact of anti-retroviral drugs? Even if their blocking effect is only modest, it should substantially reduce amplification. For example, for six reinfection cycles per burst and a 2-fold reduction in local reproduction ratio, productively infected cells will be reduced 64-fold. This reduction, we suggest, constitutes phase I. It stops when most of the local amplification processes initiated before treatment subside.

The effect of current drugs on the frequency of bursts is expected to be less profound. Initial cell-to-cell infection events are harder to inhibit than transmission at a distance[7,12] and the limited inhibitory effect on initiation is not amplified. It should result in further decrease in viral load, although at a slower rate.

Phase II: decreasing frequency of infection bursts

Decreasing immune activation in treated individuals and reduced supply of newly infected memory lymphocytes account for a progressive decrease in the frequency of infection bursts.

Unlike the primary immune response to HIV, anti-HIV re-

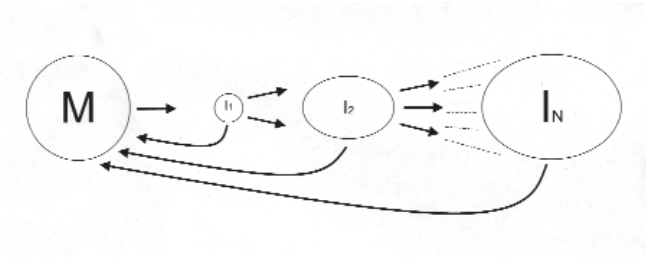


Fig. 3 Linking HIV production to immune activation. M represents a pool of infected (resting) memory cells. After activation, such cells become productively infected cells, I1, producing virus that infects recently activated cells, giving rise to I2 and so on. Ij, with j between 1 and n, represents the number of all the cells infected productively in the j's round of multiple infection processes consisting each of n sequential steps. Such processes are associated with local, transient immune responses. In parallel, a fraction of the activated cells are latently infected. Arrows pointing right, infection; arrows pointing left, flow of latently infected cells into M; circles of increasing size, amplification in the number of infected cells in each round.

sponses during the chronic phase are highly restricted, either because newly infected CD4 cells are rapidly killed by CTLs (ref. 14) or because most uninfected HIV-specific CD4 T cells are protected by anergy[12]. Generalized immune activation in HIV infection probably involves activation bursts in response to HIV as well as other persistent infectious agents that normal individuals control efficiently. The rapid removal of HIV during phase I should lead to a considerable diminution in antigenic stimulation, lowering the frequency of activation-and-infection bursts, further reducing HIV load and stimulation level.

During phase I, infection bursts are considerably reduced in size. Therefore, the output of infected memory cells should diminish, reducing the infected memory pool. The frequency of local infection bursts initiated by activation of these cells will consequently diminish, further decreasing the pool's size.

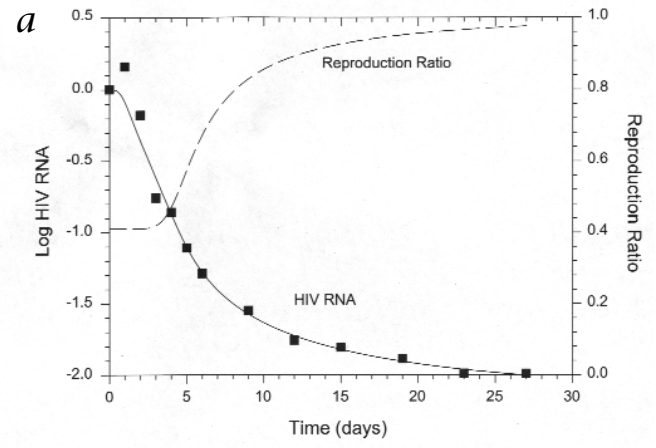
Attenuating decrease of viral load

Normalized HIV RNA measurements for 16 patients treated with a four-drug regimen showed that the rate of decrease in phase II was not only lower than that in Phase I but decreased with time (Fig. 4). A partial explanation is that infected memory cell populations with higher turnover rates contract more rapidly. Before treatment, most HIV probably emanates from clones specific for persistent infectious agents; the proportion of such HIV-infected memory cells decreases during treatment. Decreasing decline means increasing R (although R remains below 1). Such increase occurs as the fraction of cells, in the total infected-memory cell population, that die each day without causing infection becomes smaller. If blocking of new infection is complete, R simply measures the change in reactivation rate of infected memory cells.

Additional factors contribute to attenuating decrease[5]: diversity of HIV, non-uniform tissue drug distribution, and non-uniform density of activated target cells.

HIV species replicating in the same host are genetically diverse[14], indicative of functional diversity, including differential drug susceptibility. Different species can coexist, since competition is limited by the local nature of viral replication. Differential susceptibility would lead to a progressively increasing proportion of relatively resistant viruses as viral load decreases, resulting in an increase in R.

Sequestered sites such as the brain, where virus replication might continue while it is almost completely blocked elsewhere, have been invoked in relation to virus eradication[4]. Non-uniform distribution can be thought of in a more general way. This broader view is based on three considerations: virus transmission from infected to uninfected cells is likely



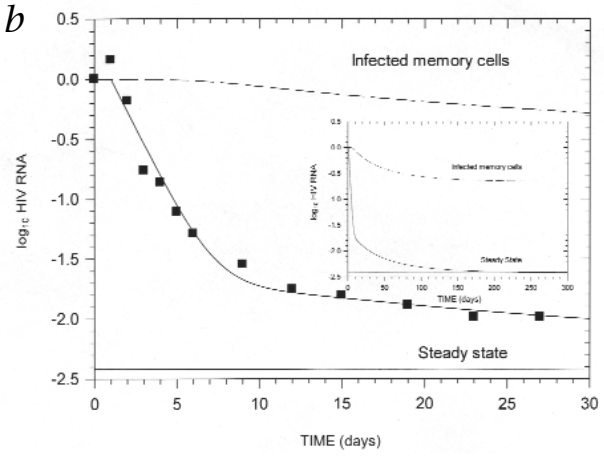


Fig. 4 HIV RNA decrease with a four-drug combination therapy. Each time point represents the geometric mean of HIV RNA from 16 patients normalized to their baseline values. Mean HIV RNA concentration at baseline: 4.0×10^4 copies/ml. ***a***, The data are shown to be consistent with a standard (but delayed cell-death) model in which R is a function of the fractional viral load (v). The equation for I_1 in Appendix 2 was rewritten as $dI_1/dt = (n/\tau)(RI_n - I_1)$ (where n = 5), with $R(v) = R(v = 1) + \alpha(1 - v)\exp(-\beta v)$. The solid line was generated by fitting the three adjustable parameters ($R(v = 1) = 0.41$, $\alpha = 0.68$, $\beta = 17$; $\tau = 1.35$ days). $R(v = 1) + \alpha > 1$, indicating the existence of a steady state. Dashed line, R as a function of time. R is reduced below 1 at treatment initiation, starts increasing when v approaches $1/\beta$, slowly approaching 1 as v further decreases . The fine details of the first few days (Fig. 1) are not reproduced in this simple approximation. ***b***, Consistency of the data with the memory cell reactivation model. The solid line was generated by simulating the model of Fig. 3 (Appendix 3). The parameters characterize frequently-reactivated clones. The effect of anti-retroviral treatment was simulated by reducing the infection-amplification factor from 3.5 to 1.3. The insert shows continued simulation to 300 days demonstrating a new steady state of HIV RNA. Productively infected cells and infected memory cells decrease at similar rates, reflecting their interdependence. (However, this does not apply to most of the latently infected cells).

Appendix 1: Reproduction ratio (R)

R is the ratio of the number of productively infected cells in a given round of infection and their number in the previous round. At the macroscopic level, cohorts of such cells replace each other continuously, although for individual cells a 'round of infection' may include latency. At steady state, R = 1; each infected cell is replaced, on average, by one newly infected cell. R is > 1 in the first weeks of acute infection and may actually increase due to increased availability of target cells generated during the anti-HIV immune response[12]. Then R decreases and assumes its (quasi-) steady-state value of about 1. This change is due to various feedback control mechanisms that affect R directly and to physiological limitation of the number of target cells[12]. Effective antiretroviral drugs initially reduce R below 1. R does not vanish when HIV transmission is completely blocked, and may even increase, because of reactivation of latently infected cells. However, in phase I, R = 0 is a good approximation in the limit of complete blocking .

Appendix 2: A macroscopic model

A variable ('distributed') time delay, from productive infection of CD4 cells to their death, is modeled by dividing the process into several equal short steps. The model's equations are[5]: $dI_1/dt = cSI_n - kI_1$; $dI_j/dt = k(I_{j-1} - I_j)$, j = 2,...*n*; $dV/dt = pI_n - qV$. The model describes in a crude way the collective behavior of many local, asynchronous, overlapping infection bursts[12]. S, average density of target cells in the vicinity of infectious cells; I_j, densities of infected cells (j, stage of infection; *n*, number of stages; only the last stage is infectious); V, virus concentration. Free virus is short-lived compared to infected cells, so that V at any time is proportional to I_n. In addition, efficient transmission from cell to cell requires close proximity. For these reasons, the rate of infection is cSI_n . The average lifespan of an infected cell is $\tau = n/k$. For constant τ, increasing *n* results in increasingly narrower distribution of time delays. In our simulations, *n* = 5. The equations here do not constitute a complete dynamical model. No equation has been specified for S. In addition, the systemic parameters c and k depend on hidden dynamic variables, reflecting the balance among functionally different cells, infected and uninfected. In particular, c is only an indirect measure of the nominal blocking efficiency of the drugs.

If the system is at steady state, $R = cS/k = 1$. When it is not at steady state, the equations can be solved analytically if it is assumed that S varies slowly enough to be treated as a parameter rather than a dynamic variable. If R < 1, the number of infected cells (and proportionally, V) quickly approaches an exponential mode of decrease. The coefficient $-\lambda$ is calculated by solving the equations for I_j using standard methods. Calculation yields the relation: $(1-\lambda\tau/n)^n = cS/k = R$. In the limit of large *n* and fixed τ, this becomes $\exp(-\lambda\tau) = R$, thus $-\lambda = \ln(R)/\tau$. The large *n* limit is already well-approximated by *n* = 5. For high treatment efficacy, when R approaches zero, the slope becomes infinite (vertical). In reality, the slope is limited by the inevitable variability in lifespan among infected cells, neglected here for simplicity.

In an infected-cell exponential death model, using $dS/dt = a - bS - cSI$, where S and I are target cell and infectious cell densities, the 'corrected' slope (in our notation) was approximately $-(\ln 2/t_{1/2})(1-c/c_0)$, where c_0 and c are infection rate coefficients before and during drug treatment, respectively[26]. The correction is small because, typically, $c/c_0 << 1$. Had we used the same equation and approximations in our delay model, the slope would be $(1/\tau)\ln(c/c_0)$, which strongly depends on treatment efficacy over a broad range of efficacies (as does $\ln(R)/\tau$). For example, if c_0 is reduced by drugs 10-fold (ref. 5) in one case and 30-fold with a different regimen, the difference in slope (with τ and $t_{1/2}/\ln 2$ both set equal to 1 day) is 1.1/day by our formula but only .046/day according to the estimate of the infected-cell half-life model.

Appendix 3: Memory cell reactivation model

Variables: M, latently infected memory cells; I_1, reactivated M-cells; I_j, productively infected cells. Equations:

$M' = -dM + bSUM(I_j)/(1+cSUM(I_j))$, j = 1,...,*n*;

$I_1' = aM/(1+c_1M) - kI_1$;

$I_j' = k(qI_{j-1} - I_j)$, j = 2,...,n.

Infected memory cells M are removed (mostly by being activated) at a rate dM (per day) and are activated in the context of multiple immune responses with aM as maximal rate. They give rise to productively infected cells, I_1, that initiate infection bursts via a chain of n productive infection cycles per burst, with amplification factor q per cycle. The backflow of newly infected memory cells into M has a maximum rate $cSUM(I_j)$, where $SUM(I_j)$ is the sum of I_j. The rates of initiation and infected memory cell regeneration are assumed to be limited at high levels of M and $SUM(I_j)$, respectively. Possible mechanisms: When M is large, there is a higher probability of premature initiation, before a sufficiently large number of target cells have been generated. The generation of latently infected cells may require contact interaction of activated CD4 T cells with infectious APCs. If the number of infectious APCs and/or activated CD4 T cells, not that of infected T cells, is limiting, the generation rate will be relatively insensitive to increase in $SUM(I_j)$. Productively infected cells are assumed here to die at a rate k, rather than by a multistep process, as now we are interested in long-term kinetics and a crude simulation of phase I decrease is good enough. In simulations, a delay τ is introduced before the above equations are applied. Fitting of the data in Fig. 4*b* is achieved with d = a = 0.1/day, k = 1, τ = 1 day, c = 1, c_1 = 0, b = 0.1, *n* = 6 and q = 3.5 before treatment and 1.3 during treatment. With these parameters, a new steady state is reached during treatment. The model did not admit self-renewal division of infected memory cells. If such renewal is allowed, a new steady state can exist for a broader range of parameter values.

to be mainly local[12]; drugs block transmission only partially; and drug tissue distribution is non-uniform. These considerations indicate that viral replication in the presence of drugs may vary considerably across tissues. The overall rate of decrease is the weighted average of all such local processes, with weights proportional to the local levels of infection. Although viral load as a whole decreases, the relative weight of tissue domains that are less accessible to drugs increases, leading to an increasing R.

Like fitter viruses and less-accessible sites, more-susceptible target cells—for example, because of a higher density[5]—are also 'selected', with a similar attenuating effect.

The kinetics of attenuating HIV decrease can be simulated by making R a function of $v = V/V_0$ (the fractional viral load relative to the baseline). For example, $R(v)=R(v=1) + \alpha(1-v)\exp(-\beta v)$, where α and β are adjustable parameters (Fig. 4*a*). R(v = 1), at treatment initiation, is < 1. R increases as v decreases and may eventually reach 1, in which case the decrease stops. Increasing R does not necessarily indicate active HIV transmission, but reaching or exceeding R = 1 does.

Long-term persistence of HIV replication

Given that some infection events are more inhibitable by drugs than others, we predicted that levels of HIV may not extinguish but approach a stable steady state[5,12]. Indeed, if R is already close to 1 after 3–4 weeks (Fig. 4*a*), R may become 1 for some finite level of virus, even if that level is currently undetectable. This idea is gaining empirical support[17–20]. Formally, the condition is that $R(v = 1) + \alpha$, the value of R as v approaches 0, should be larger than 1, indicating that R equals 1 for some v > 0.

Thus, attenuating decrease might reflect stabilization of the number of infected cells. Such stabilization occurs, in part, as immune activation returns toward normal. It may also reflect readjustment of immunologic control mechanisms, such as anti-HIV CTLs (ref. 21) and protective anergy[12,22], to reduced

antigenic stimulation. In addition, as the total number of frequently activated infected memory cells diminishes, their replenishment may increasingly compensate for their death until balance is restored. This is demonstrated with a simple mathematical model (memory-cell reactivation model; Appendix 3 and Fig. 3) that fits the data (Fig. 4*b*).

Our models suggest that among treatment regimens that initially reduce R below 1, those that are less efficient (R(v = 1) closer to 1) would result in higher levels of virus in the new steady state. A less effective regimen, such as highly active anti-retroviral therapy (HAART) administered to individuals with a previous history of non-optimal anti-retroviral therapy, provided an opportunity to test this. Viral loads were measured in a group of adults and a group of children who had been treated previously and who responded to the new treatment regimens by at least a 1-log decrease in plasma HIV RNA. Among those adults in whom HIV RNA remained dectable, the average slope over a 7- to 20-month period beginning 3–5 months after the start of the new treatment was $-2 \times 10^{-5} \pm 0.03$ (mean ± s.d.), a value not significantly different than zero (data not shown). In the children, slopes were estimated for a period of 44–68 weeks beginning 26 weeks after changing to HAART. The average slope was also not different from zero (0.0003 ± 0.02) (data not shown). Given the broad range of levels at which viral burden can stabilize, down to the limit of detectibility, individuals with 'undetectable levels' might also have stable rather than declining HIV burdens.

An insight into this issue is provided by the ADAM study[23], in which patients were treated for 26 weeks with a four-drug regimen; half of them were then put on a two-drug 'maintenance' regimen. Ten weeks later, 9 of 14 patients in the maintenance group had detectable plasma HIV RNA (mean, 289 copies/ml), whereas only 1 of 11 patients in the other group did.A conventional explanation for these findings is a higher frequency of virus escape mutations during the maintenance therapy phase. Based on two consecutive measurements during a 4-week period in three patients and three measurements during 10 weeks in one patient, the rate of increase in HIV RNA was very low (82 particles/ml per week), unlike the rapid rebound expected from emergence of resistant mutants. Two larger trials have confirmed these findings[24,25]. In a group of patients on a reverse-transcriptase/protease maintenance protocol, no essential mutations associated with drug resistance were found in 24 of 25 'failure cases' (D. Descamps *et al.*, Abstract 493, Sixth Conference on Retroviruses and Opportunistic Infections, Chicago, Illinois,1999).

Our interpretation is that in most maintenance 'failures' the viral load increases from below detectibility towards a new steady state, simply as a consequence of the relationship, predicted in our model, between the steady-state level and treatment efficacy. We suggest that, had these patients been on the two-drug regimen from the beginning, they would have reached a detectable viral load without it first decreasing below detectibility.

Summary and implications

We propose that reduced virus production during immune activation events, and fewer infection cycles, account for the observed multiphasic HIV decrease and for viral persistence in treated individuals. We have presented data supporting this thesis that are inconsistent with the premise that viral replication under HAART is insubstantial, except in isolated tissue domains, load decreases at the rate at which different sets of previously infected cells die.

Our interpretation is based on the close relationship between virus transmission and immune activation. One need not postulate distinct populations with different homogeneous behaviors; instead, the pattern of viral decrease reflects physiologically structured, local non-equilibrium interactions between HIV and immune activated cells after treatment initiation. The shrinking population of infected cells manifests an increasing average reproduction ratio, resulting in a decreasing rate of viral load decrease and in the possibility of reaching a stable level of viral replication in treated individuals.

Our model indicates that eradicating HIV by drugs alone will be a formidable task. Indeed, the idea that HIV is produced in multiple, hard-to-abolish, local bursts, whose size is very sensitive to relatively small changes in blocking efficiency, implies that the efficacy of drug combinations available at present has been overestimated. Therefore, stimulating all latently-infected cells to produce virus under the cover of HAART, with the goal of eradication, may actually result in transmission to new cells. In such a strategy, stimulation would need to be coupled with additional means to eradicate infected cells, such as enhancing anti-HIV CTL activity or neutralizing antibodies. However, anti-retroviral drugs with higher efficacy than those now available may maintain $R < 1$ across all tissue domains, effectively terminating transmission and making eradication a possibility.

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